

1 DIFFERENCES IN THE CHEMICAL COMPOSITION OF ORGANIC-WALLED  
2 DINOFLAGELLATE RESTING CYSTS FROM PHOTOTROPHIC AND  
3 HETEROTROPHIC DINOFLAGELLATES<sup>1</sup>

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30 *Condensed running title*  
31 Dinoflagellate cyst compositional differences

## ABSTRACT

Dinoflagellates constitute a large proportion of the planktonic biomass from marine to freshwater environments. Some species produce a preservable organic-walled resting cyst (dinocyst) during the sexual phase of their life cycle that is an important link between the organisms, the environment in which their parent motile theca grew, and the sedimentary record. Despite their abundance and widespread usage as proxy indicators for environmental conditions, there is a lack of knowledge regarding the dinocyst wall chemical composition. It is likely that numerous factors, including phylogeny and life strategy, determine the cyst wall chemistry. However, the extent to which this composition varies based on inherent (phylogenetic) or variable (ecological) factors has not been studied.

To address this, we used micro-Fourier transform infrared (FTIR) spectroscopy to analyze nine cyst species produced by either phototrophic or heterotrophic dinoflagellates from the extant orders Gonyaulacales, Gymnodiniales and Peridiniales. Based on the presence of characteristic functional groups, two significantly different cyst wall compositions are observed that correspond to the dinoflagellate's nutritional strategy. The dinocyst wall compositions analyzed appeared carbohydrate-based, but the cyst wall produced by phototrophic dinoflagellates suggested a cellulose-like glucan, while heterotrophic forms produced a nitrogen-rich glycan. This constitutes the first empirical evidence nutritional strategy is related to different dinocyst wall chemistries. Our results indicated phylogeny was less important for predicting composition than the nutritional strategy of the dinoflagellate, suggesting potential for cyst wall chemistry to infer past nutritional strategies of extinct taxa preserved in the sedimentary record.

## *Keywords*

dinoflagellate cyst, dinosporin, heterotrophic, infrared spectroscopy, macromolecule, nutritional strategy, phototrophic

58

59 *Abbreviations*

60 FTIR, Fourier transform infrared

61 DCM, dichloromethane

62 EtOH, ethanol

63 Pyr-GC-MS, pyrolysis gas chromatography-mass spectrometry

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## INTRODUCTION

Dinoflagellates are biflagellate, eukaryotic protists that comprise a large proportion of planktonic biomass (Taylor 1987) and are therefore important components of marine and freshwater ecosystems. Some species, as part of their sexual reproduction and in preparation for a dormant period in their life cycle, produce an organic-walled, refractory resting cyst (Wall and Dale 1968, Pfister and Anderson 1987, Head 1996) capable of being preserved in the sediment record. The existence of two life cycle stages has resulted in the creation of two taxonomic systems: one used by biologists and based on the morphology of living motile cells, and the other developed by paleontologists and based on resting cyst morphology. Dinoflagellate resting cysts (dinocysts) are important sources of environmental information for modern marine (e.g., Rochon et al. 1999, Dale et al. 2002, Matthiessen et al. 2005, Holzwarth et al. 2007, Pospelova et al. 2008, Bouimetarhan et al. 2009, Holzwarth et al. 2010, Zonneveld et al. 2013, Bringué et al. 2013) and freshwater (e.g., Kouli et al. 2001, Tardio et al. 2006, Leroy et al. 2009, McCarthy et al. 2011, Mertens et al. 2012) ecosystems, and are also valuable for the reconstruction of oceanographic conditions (e.g., Reichart and Brinkhuis 2003, Pospelova et al. 2006, González et al. 2008). They therefore represent the link between organisms, the environment, and the sedimentary record. However, dinocyst taxa react in different ways to oxidation; cysts from the order Peridiniales show high sensitivity, while cysts from the order Gonyaulacales demonstrate greater resistance, both in laboratory and natural settings (Dale 1976, Zonneveld et al. 1997, 2001, Combourieu-Nebout et al. 1998, McCarthy et al. 2000, Hopkins and McCarthy 2002, Mertens et al. 2009a). This suggests that intrinsic properties of the dinocyst walls vary between the different orders, some physical, such as differences in wall thickness or structure, while others are chemical. In this study, we focused on potential differences in cyst wall chemistry.

To date, little is known regarding the macromolecular composition of dinocyst walls, which is comprised of a refractory biomacromolecule referred to as dinosporin (Fensome et

al. 1993). Different compositions have been suggested, including mainly aromatic (Kokinos et al. 1998), a mixture of aromatic and aliphatic (Hemsley et al. 1994), and mainly aliphatic and heavily cross-linked (Versteegh et al. 2007). More recently, a carbohydrate-based dinosporin has been suggested on the basis of Fourier transform infrared (FTIR) analyses and pyrolyses of *Lingulodinium machaerophorum* (Deflandre and Cookson) Wall from culture and surface sediments (Versteegh et al. 2012). Further micro-FTIR spectroscopic analyses of late Paleocene dinocysts in the genus *Apectodinium* (Costa and Downie) Lentin and Williams agreed with this interpretation but also displayed considerable differences in composition between species of the same genus (Bogus et al. 2012). Therefore, it seems that phylogeny could be a factor contributing to dinosporin compositional differences. However, there are also other factors with significant potential to influence dinosporin composition, including differences in the compounds available within the motile dinoflagellate cell which builds the cyst, and differences resulting from post-depositional alteration. It is assumed that dinoflagellates build their cysts from material readily available within the cell (Kokinos and Anderson 1995, Hallet, 1999), which was suggested by TEM studies of cyst formation in the freshwater species *Ceratium hirundinella* (Müller) Dujardin (Chapman et al. 1982). Furthermore, there is clear evidence that dinocyst morphology can vary with differing environmental conditions (e.g., Hallet 1999, Ellegaard 2000, Lewis et al. 2001, Ellegaard et al. 2002, Zonneveld and Susek 2007, Mertens et al. 2009b) as well as evidence that some dinoflagellates, such as *Karlodinium veneficum* (Ballantine) Larsen and *Alexandrium minutum* Halim (Fuentes-Grünwald et al. 2009, 2012) can adapt their cellular composition to different environmental conditions. However, it is not known whether there is a link between chemical compounds available within the motile cell and dinosporin composition. One way to investigate potential impacts on dinosporin chemistry is to analyze dinocysts produced by dinoflagellates that have very different life strategies, such as nutritional strategies (phototrophy versus heterotrophy). Additionally, diagenetic processes, such as natural

vulcanization, have been shown to chemically alter the cyst walls of *Thalassiphora pelagica* (Eisenack) Benedek and Gocht emend. (Versteegh et al. 2007) and raised the question as to what extent the differences observed between *Apectodinium* species were influenced by differential diagenesis (Bogus et al. 2012). However, we circumvented the diagenetic factor by utilizing specimens isolated from modern marine and lacustrine surface sediments where the exposure to diagenetic processes is short and thus expected to be small.

We investigated differences in the dinocyst wall chemistry in species from different lineages (the orders Gonyaulacales, Gymnodiniales, and Peridinales). The main objectives were to determine the primary factors that affect dinosporin composition and to address the extent to which a particular dinocyst composition was monophyletic, i.e., different dinoflagellate lineages produce different wall compositions, or polyphyletic and subject to variations resulting from different nutritional strategies, since these are known to be polyphyletic. The dinocysts were analyzed using micro-FTIR spectroscopy. This versatile technique has already been successfully used to identify compounds in the complex biopolymers of dinocyst wall layers of optically identified and individually isolated specimens (e.g., Kokinos et al. 1998, Versteegh et al. 2007, 2012, Bogus et al. 2012).

## MATERIAL AND METHODS

### *Sample treatment*

Samples were retrieved from marine and lacustrine locations (Fig. 1, Table 1). Surface sediment samples from the marine Benguela upwelling region (GeoB 2341, 4804), off the coast of northwest Africa (GeoB 6010), and freshwater Honey Harbour in Lake Huron, Ontario, Canada (SV5-C) were briefly ultrasonicated (< 60 s) in Milli-Q water to disaggregate particles attached the cyst walls, sieved over a 50 µm nylon mesh sieve and retained on a 20 µm precision sieve (Storck Veco #317). Other specimens of empty dinocysts, previously isolated for germination experiments, were analyzed from the Wadden Sea (NW Germany)

and Omura Bay (Kyushu, Japan). The various geographic locations, environmental settings and isolation procedure (i.e. empty cysts isolated from the sediment matrix or empty cysts utilized shortly after hatching) were used to verify that these different situations did not overprint the cyst wall signal.

To remove extraneous apolar contaminants, all material was soaked in ethanol (EtOH) or dichloromethane (DCM) for at least 30 min. To remove extraneous water soluble molecules, all material was then rinsed three times with Milli-Q water. Similar to the procedure described in Versteegh et al. (2007) and Bogus et al. (2012), individual specimens with no visibly attached particles were identified to species level with a light microscope, isolated with a micropipette, transferred to either a salt (NaCl) plate or an Au-coated mirror, dried overnight at 60 °C, and analyzed immediately.

In total, specimens from nine extant dinocyst species from the orders Gonyaulacales, Gymnodiniales, and Peridinales were isolated. The cyst species are produced by either phototrophic or heterotrophic dinoflagellates. Cyst names are used in the descriptions. At least three specimens of each species were picked and analyzed, and in the case of two species, specimens from two different locations were analyzed (Table 2).

#### *Micro-Fourier transform infrared spectroscopy*

Infrared spectra of specimens from GeoB 2341, GeoB 4804, and GeoB 6010 were recorded with a Nicolet FT-IR spectrometer coupled to a Nicplan microscope, a Protégé™ 460 optical bench, a mercury cadmium telluride (MCT)- A detector cooled with liquid N<sub>2</sub>, Ever-Glo source, and a KBr beamsplitter. The adjustable apertures (upper and lower) were set at a constant area of 15 x 15 µm. Two hundred and fifty-six scans at 8 cm<sup>-1</sup> resolution were obtained in transmission mode over a spectral range of 4000-650 cm<sup>-1</sup>. All other specimens were analyzed with a BRUKER IFS 66v coupled to an IR Scope II equipped with a MCT detector cooled with liquid N<sub>2</sub> and KBr beamsplitter. Two hundred and fifty-six scans at 4



cm<sup>-1</sup> resolution were recorded in reflection mode over a spectral range of 4000-650 cm<sup>-1</sup>. The replicability of FTIR analysis using these two different devices was checked through the use of a chitin standard (Sigma, Lot 59F7265). The spectra proved consistent and are displayed after subtracting the background of air and the bare plates (NaCl [Nicolet]; Au [Bruker]), and baseline correction. Assignments of the main IR absorptions to chemical bonds (Table 3) were based on Colthup et al. (1990), Coates (2000), and additional published literature.

## RESULTS AND DISCUSSION

### *Evidence for carbohydrate-based dinosporins*

For all species, the region between 3600-3000 cm<sup>-1</sup> showed a strong and broad absorption with a maximum near 3350 cm<sup>-1</sup> (OH stretch) and, sometimes, a shoulder near 3270 cm<sup>-1</sup> and/or 3100 cm<sup>-1</sup> (Table 3, Figs. 2 and 3). Absorptions were relatively weak in the 3000-2775 cm<sup>-1</sup> region (CH stretching), except for the spectrum of *Impagidinium patulum* (Wall) Stover and Evitt, which exhibited two stronger absorptions at 2925 cm<sup>-1</sup> and 2860 cm<sup>-1</sup>. Since this was observed in all *I. patulum* specimens, it is likely an intrinsic component. The relatively weak absorptions in this region suggested a minor contribution from CH<sub>2</sub> and CH<sub>3</sub> groups. There was a pattern of four absorptions between 1200-1030 cm<sup>-1</sup> that are highly indicative of C-O stretching and the deformation vibrations of sugar rings: 1160 cm<sup>-1</sup> (C-O-C asymmetric vibration), 1110 cm<sup>-1</sup> (glucose ring stretch), 1060 cm<sup>-1</sup> (C-O stretch), and 1030 cm<sup>-1</sup> (C-O stretch). This series was most apparent in *Dubridinium caperatum* Reid, *Spiniferites pachydermus* (Rossignol) Reid, *Tuberculodinium vancampoae* (Rossignol) Wall, cysts of *Polykrikos schwartzii* Bütschli, and cysts of *Peridinium wisconsinense* Eddy. The remaining species, *Operculodinium centrocarpum* sensu Wall and Dale, *I. patulum*, *Brigantedinium* spp. Reid, and cysts of *Polykrikos kofoidii* Chatton, exhibited most of these absorptions; however, the appearance of this region either differed by containing less definition, as in *O.*

*centrocarpum*, *I. patulum*, and *Brigantedinium* spp., or by appearing shifted, as in cysts of *P. kofoidii*. This variability is discussed further in subsequent sections.

Despite the variability exhibited, the absorptions between 1200-1030 cm<sup>-1</sup>, together with the diagnostic absorptions at 896-902 cm<sup>-1</sup>, provide a strong argument that a carbohydrate with a  $\beta$ -glycosidic linkage (Kačuráková and Wilson 2001, Cárdenas et al. 2004, Versteegh et al. 2012) forms the backbone of dinosporins. The cell walls of the motile stage of the freshwater dinoflagellate *Peridinium westii* Lemmermann were shown to contain both  $\beta$ -1 $\rightarrow$ 3 and  $\beta$ -1 $\rightarrow$ 4 glycosidic bonds (Nevo and Sharon 1969). Unfortunately, the FTIR spectra of  $\beta$ -1 $\rightarrow$ 3 linked glucans (e.g., Furuhashi et al. 2009) can be similar to those with  $\beta$ -1 $\rightarrow$ 4 linkages (e.g., Pandey 1999) that are also apparent in the highly resolved 1000-600 cm<sup>-1</sup> region described by Barker et al. (1954). It is therefore not currently possible to definitively determine the type of  $\beta$ -glycosidic linkage in the dinosporin macromolecule. Regardless, the analyzed species demonstrate carbohydrate-based dinosporins and complement the interpretation of a carbohydrate backbone from FTIR spectroscopy and pyrolysis gas-chromatography mass spectrometry of culture- and sediment-derived *Lingulodinium machaerophorum* (Versteegh et al. 2012) as well as FTIR analyses of the extinct genus *Apectodinium* (Bogus et al. 2012).

#### *Evidence for two broadly different dinosporin groups*

Regardless of the evidence for a carbohydrate-based dinosporin composition in all dinocyst species, it appears that at least two broadly different types of dinosporin occur. This distinction was based on the pattern of absorptions between 1850-650 cm<sup>-1</sup> (Figs. 2 and 3) and evidence of the main functional groups present (Table 3). The first dinosporin group, here referred to as Group I, includes *Impagidinium patulum*, *Operculodinium centrocarpum*, *Spiniferites pachydermus*, *Tuberculodinium vancampoe* (all Gonyaulacales) and the freshwater cysts of *Peridinium wisconsinense* (Peridinales; Fig. 2). In this group,

absorptions between 1200-1030  $\text{cm}^{-1}$  are dominant, absorptions between 1470-1200  $\text{cm}^{-1}$  ( $\text{CH}_2$  and  $\text{CH}_3$  bending and rocking; OH in-plane deformation vibrations) are second in amplitude, and those between 1850-1600  $\text{cm}^{-1}$  (adsorbed OH; conjugated C=O bonds) are the weakest. The only exception was in the spectrum of *T. vancampoe*, where the absorption at 1600  $\text{cm}^{-1}$  is almost as strong as the 1200-1030  $\text{cm}^{-1}$  region.

There was spectral variability within Group I, such as the changes in relative intensity between the 1640  $\text{cm}^{-1}$  and 1600  $\text{cm}^{-1}$  absorptions (Fig. 2). The most prominent 1640  $\text{cm}^{-1}$  absorption is exhibited in the *S. pachydermus* spectrum, whereas it is either a shoulder or a weak peak in the other species. This pattern is reversed for the absorption at 1600  $\text{cm}^{-1}$  where it is only a shoulder in *S. pachydermus*, but a stronger signal in *O. centrocarpum* and, especially, *T. vancampoe*. A strong peak at 1600  $\text{cm}^{-1}$  could suggest more ester bonds in those species' cyst walls (e.g., Yuen et al. 2009). However, minor absorptions between 3000-2800  $\text{cm}^{-1}$  and 1470-1350  $\text{cm}^{-1}$  imply that methylene and methyl groups, and thus ester bonds, cannot be responsible for a more pronounced absorption at 1600  $\text{cm}^{-1}$ . It is also possible that this represents the influence of aromatic skeletal vibrations, as shown in lignin (Pandey 1999), but there is no clear evidence for aromatic vibrations ( $\sim 1500 \text{ cm}^{-1}$ ) in any of the dinocyst spectra. Therefore, at this point, a more definitive explanation of the 1600  $\text{cm}^{-1}$  absorption is not available.

There was also variability in the deformation pattern between 1200-850  $\text{cm}^{-1}$ . This region is comprised of four separate, defined absorptions in the cysts of *P. wisconsinense*, *S. pachydermus*, and *T. vancampoe* that closely match the spectrum of the  $\beta$ -linked glucan, cellulose (Fig. 2) as well as the previously published spectrum for *Lingulodinium machaerophorum* (Versteegh et al. 2012). In fact, the spectrum of *S. pachydermus* was so remarkably similar to cellulose overall that each of the absorptions observed for *S. pachydermus* can easily be assigned using the cellulose spectrum (Pandey 1999). The lack of this absorption series in *I. patulum* and *O. centrocarpum*, particularly the peak at 1112  $\text{cm}^{-1}$ ,

and the existence of a broader absorption centered at  $1060\text{ cm}^{-1}$  may indicate that glucose is not the only sugar monomer present. While cellulose is the best known  $\beta$ -glucan (Aspinall 1983) and is described as the primary material comprising the theca of dinoflagellates (Sekida et al. 2004), other non-cellulosic  $\beta$ -glucans, such as mannan, are common and well-documented components in plant (e.g., Kačuráková and Wilson 2001, Burton and Fincher 2009) and algal cell walls (e.g., Frei and Preston 1964, Stone 2009), including the motile dinoflagellate *Peridinium westii* (Nevo and Sharon 1969). Thus, it is likely that non-cellulosic  $\beta$ -glucans also contribute to the carbohydrate signal of dinosporins in this group. In general, we propose that a spectral signal indicating  $\beta$ -glucans represents the signature composition of these dinocyst species.

Dinocysts in the second group (Group II) consist of *Brigantedinium* spp., *Dubridinium caperatum* (both Peridinales), and cysts of *Polykrikos schwartzii* and *P. kofoidii* (Gymnodinales). These species exhibited a different spectral pattern of relative absorption strength, demonstrated greater heterogeneity with respect to the relative intensity of each of the regions, and, most significantly, included evidence for nitrogen (N)-containing functional groups (Fig. 3, Table 3). The region between  $1850\text{-}1500\text{ cm}^{-1}$  dominated in *D. caperatum* and cysts of *P. kofoidii*, but it was less intense in cysts of *P. schwartzii* and *Brigantedinium* spp. Within this region, maxima occurred especially between  $1585\text{-}1560\text{ cm}^{-1}$ , and there was a clear shoulder at  $1660\text{ cm}^{-1}$ . Absorptions between  $1585\text{-}1560\text{ cm}^{-1}$  are characteristic of amide II bonds (CN stretching and NH bending), while the shoulder at  $1660\text{ cm}^{-1}$  in all of the species probably reflects amide I bonds, which is the result of the influence of hydrogen bonding ( $\text{C}=\text{O}\cdots\text{H}-\text{N}$ ; Cárdenas et al. 2004). The area between  $1500\text{-}1200\text{ cm}^{-1}$  dominated only in *Brigantedinium* spp., although all of the Group II dinocysts showed a small absorption near  $1255\text{ cm}^{-1}$  (NH bending). The absorptions between  $1420\text{-}1370\text{ cm}^{-1}$  reflect CH bending, and the absorption at  $1312\text{ cm}^{-1}$  indicates CN stretching and NH bending (amide III). Further evidence for the presence of nitrogen included a shoulder at  $3100\text{ cm}^{-1}$  (NH stretching),

further NH stretching absorptions ( $\sim 3268\text{ cm}^{-1}$ ) likely encompassed within the broad OH stretching region ( $3600\text{-}3000\text{ cm}^{-1}$ ) and manifested by a shifting of the peak center (relative to Group I), and a small peak at  $698\text{ cm}^{-1}$  (NH wagging; amide V). Absorptions between  $1200\text{-}1030\text{ cm}^{-1}$  (C-O stretching) account for a much smaller proportion of the total absorptions from  $1850\text{-}830\text{ cm}^{-1}$  with the exception of cysts of *P. schwartzii*, where the absorptions in this region dominated and clearly demonstrated the four separate absorptions characteristic of sugar ring vibrations present in many of the Group I dinocysts. In combination, the spectral evidence in Group II included many absorptions that are typically seen in the spectrum of the polysaccharide chitin (Fig. 3a; e.g., Cárdenas et al. 2004) as well as peptides (e.g., Venyaminov and Kalnin 1990). The evidence of N-containing functional groups may reflect single amino acids, as is the case for chitin, or more complex, proteinaceous material such as (oligo)peptides. The combination of the carbohydrate evidence, together with the amide bond evidence, suggest a dinosporin composition based on a more chitin-like glycan (Stankiewicz et al. 1998, Kačuráková et al. 1999, Cárdenas et al. 2004) or even a chitin-glucan complex (Šandula et al. 1999). The exhibited in-group variability could indicate different sugar moieties, as postulated for Group I, suggest contributions of different amino acids (Venyaminov and Kalnin 1990) or chitins (i.e.  $\alpha$  or  $\beta$ , Cárdenas et al. 2004), and/or reflect varying ratios of N-containing functional groups to the carbohydrate backbone.

#### *Explanation of compositional differences*

Our results demonstrate considerable spectral and, thus, compositional diversity among the dinocyst species that indicate dinosporin is a chemically heterogeneous compound. The most fundamental distinction between the dinosporins is the inclusion of N-containing functional groups in Group II and we now explore the reasons for this difference in composition. Each of the dinocyst species in Group I is produced by phototrophic dinoflagellates, while the Group II dinocysts are produced by heterotrophic dinoflagellates (Table 2). The heterotrophic

species studied here prey upon a variety of dinoflagellates and diatoms (e.g., Jacobson and Anderson 1986, Matsuoka et al. 2000, Naustvoll 2000, Menden-Deuer et al. 2005). Therefore, the origin of the amide groups in Group II dinosporins may be from predation by the dinoflagellates, which leads to an accumulation of N-rich compounds (i.e., proteinaceous compounds) within the cell as a result of prey digestion. Many heterotrophic organisms, whose growth is energy limited, produce cell coverings that contain both amino acids and sugars like peptidoglycans (bacteria) and chitin (arthropods and fungi) because both types of compounds are abundant in prey and therefore energetically favorable to use. On the other hand, phototrophic organisms are not energy limited, but nutrient limited (i.e. nitrogen and phosphorus). Therefore, it is not energetically favorable for them to utilize these limited nutrients to build a metabolically inactive cell covering, but rather to incorporate the products of photosynthesis (e.g., Thornton et al. 1999, Wotton 2004, Ellegaard et al. 2013). As dinocyst walls are assumed to be constructed using compounds from within the dinoflagellate cell (Kokinos and Anderson 1995), the incorporation of photosynthetic products into a Group I dinocyst would account for a glucan backbone, while the predominance of proteinaceous material would lead to the inclusion of N-containing functional groups into Group II dinocysts. In both cases, dinoflagellates thus use compounds that are in excess and energetically favorable, determined by their nutritional strategy.

Compositional differences between phototrophic and heterotrophic dinoflagellates and their cysts have previously been suggested by studies investigating the autofluorescence of the two groups. Fluorescence microscopy was used to distinguish motile photosynthetic dinoflagellates from heterotrophic ones (Lessard and Swift 1986). Additional work with fossil and recent heterotrophic dinocysts demonstrated they do not exhibit autofluorescence (Brenner and Biebow 2001) and this absence has been used to infer a heterotrophic ecology in newly described dinocyst species (Verleye et al. 2011). Our results support these findings by

providing the first description of differences in chemical composition between phototrophic and heterotrophic dinocysts.

In contrast to the expectation that dinocyst composition might solely exhibit characteristic phylogenetic differences, a clear division along phylogenetic lines is absent. Most of the Group I dinocysts are in the order Gonyaulacales, while Group II dinocysts are either Peridinales or Gymnodinales; however, cysts of *Peridinium wisconsinense* exhibit spectra firmly positioned in Group I despite being in the order Peridinales. Both compositional groups are polyphyletic when compared to dinoflagellate phylogeny (Fensome et al. 1993, Taylor 2004, Zhang et al. 2007, Hoppenrath and Leander 2010, Orr et al. 2012; Fig. 4). Therefore, we propose that nutritional strategy rather than phylogeny is the primary factor identified which determines cyst wall composition. However, related species tend to have related life strategies so some covariance with species phylogeny is expected. This is most evident in Group II where the spectra of the *Polykrikos* species are more similar in terms of relative absorption intensities than to the other taxa investigated in this group (Fig. 3).

We cannot exclude the possibility that some of the differences observed result from taphonomic and environmental heterogeneity and/or preparative processes. Taphonomic processes, such as sulfurization in anoxic environments (Kok et al. 2000, van Dongen et al. 2003, Versteegh et al. 2007) or oxidative polymerization in oxygenated settings (Versteegh et al. 2004, Gupta et al. 2006, de Leeuw 2007), can overprint the original biomacromolecular signal. Even though the analyzed specimens were isolated from surface sediments, they may have undergone some early diagenetic alteration that led to the loss of the most easily degradable components. Diagenetic effects on dinosporin composition are not well known, but Versteegh et al. (2007) showed an initial preferential loss of oxygen-containing functional groups as well as an increase in aliphatic CH<sub>x</sub> bonds during diagenesis. All of our measured specimens demonstrated strong absorptions for oxygen-containing functional groups and

relatively weaker absorptions for aliphatic CH<sub>x</sub>, suggesting that diagenesis as a whole has not significantly affected the cyst wall chemistry.

Some of the variability seen between and within the two groups may also be explained by the presence, absence and/or thickness of layers with different contributions of carbohydrates and amino acids, or it may be that different proportions of these compounds remain attached to the cyst wall after hatching or migrate onto the cyst wall from the sediment. However, there was clear consistency between spectra of the same dinocyst species measured from more than one location, *Operculodinium centrocarpum* and cysts of *Polykrikos schwartzii* (Table 2, Figs. 2, 3 [gray lines]). The distinction between Group I and II dinosporins was also consistent between empty cysts of *Tuberculodinium vancampoe* and *Dubridinium caperatum* measured shortly after hatching from. The maintenance of this distinction (i.e. the absence of nitrogen-containing functional group evidence in *T. vancampoe*) indicates little effect, if any, from leftover cell material after sample treatment.

In terms of preparative alteration, the specimens analyzed all represent visibly and chemically clean, but thermally untreated material. Therefore, the origin of the amide bonds cannot be artifacts, such as melanoidin-like polymers, produced during preparation (Allard et al. 1997, 1998). Cleaning involved gentle ultrasonic treatment (< 60 s; Mertens et al. 2009a) and solvent extraction, specifically washing with Milli-Q water to remove water soluble contaminants, and DCM/EtOH to remove apolar contaminants. The use of solvent extraction alone, as opposed to its use in conjunction with acid and/or base hydrolysis (e.g., Kokinos et al. 1998, Versteegh et al. 2012), was used for a couple of reasons. First, even a short treatment with a base selectively destroys peridinialean cysts (e.g., Dale, 1976, Mertens et al. 2009a). Additionally, a comparison between extracted and hydrolyzed cultured *Lingulodinium machaerophorum* cysts and extracted sediment-derived cysts showed remarkably similar spectra (Versteegh et al. 2012), suggesting that hydrolysis was not necessary to render the cysts chemically clean. The *L. machaerophorum* spectra are very consistent with the Group I



spectra (Fig. 2). It may not be possible to fully discount effects of invisible material on/in cyst walls insoluble in water and organic solvents on resulting spectra, particularly for species with significant surface ornamentation such as *O. centrocarpum*, *T. vancampoeae*, *Spiniferites pachydermus* and the *Polykrikos* species, but the lack of evidence for an overprint indicates this is unlikely; instead, it suggests that preparing cysts with solvent extraction has the potential for wide applicability as even delicate cysts with lower resistance to harsh chemical treatments will be able to be reliably analyzed.

The aforementioned observations indicating (1) little to no diagenetic effects, (2) consistency between the same species analyzed from multiple locations as well as the maintenance of the group-wise distinction between different species of freshly hatched cysts, and (3) the similarity of the Group 1 spectra to spectra from hydrolyzed *L. machaerophorum* cysts argue against any significant overprint from diagenesis, environment and sample preparation.

#### *Implications for (paleo)environmental studies*

A determination of two broadly different dinosporin types was based on the FTIR spectral patterns and functional groups present, and attributed to nutritional strategy. The spectral variability, particularly with regards to the relative strength of the individual absorptions, suggests that the story is likely more complicated given the large variety in nutritional strategies amongst dinoflagellates (Schnepf and Elbrächter 1993) as well as previous evidence that aspects of dinosporin composition are also likely taxon-specific (de Leeuw et al. 2006, Bogus et al. 2012, Figs. 2, 3). So, the spectral variability within the two groups does not exclude a phylogenetic component in dinosporin synthesis, only that it is obscured in this case by the complex interplay between the dinoflagellate and its environment. Thus, it may be more accurate to define dinosporin overall as a suite of chemically distinct but related carbohydrate-based biomacromolecules. The most apparent distinction in a broad sense is the

inclusion of amide groups into some species' dinosporins, resulting from heterotrophy, and in this sense dinosporin does not follow a strict delineation with phylogeny (Fig. 4). Therefore, based on the evidence, we suggest FTIR spectra of dinocysts have the potential to assess (paleo)nutritional strategies. This may prove especially interesting for species that may change their nutritional strategy in response to environmental conditions.

Very important subsequent steps are to expand the number of investigated taxa in general and through the sedimentary record to robustly determine the predictive value of dinosporin composition for (paleo)ecology. Even though carbohydrates are the most abundant form of biomass on earth (Kurita 2006), they are usually considered labile compounds (e.g., de Leeuw and Largeau 1993, Arnosti 1995) with preservation determined by the lability of individual sugar monomers (Moers et al. 1994, Marchand et al. 2009) and diagenetic alteration, such as macromolecular skeletal rearrangements (Almendros et al. 1997). There is evidence for carbohydrate preservation relatively far back in the sedimentary record ([Miocene] e.g., Lechien et al. 2006), including well-preserved glycolipids ([Eocene] Bauersachs et al. 2010) as well as spectra demonstrating good preservation of Group I compositional signals ([late Paleocene] Bogus et al. 2012). Dinospore is likely to involve some inherent significant structural differences to straightforward carbohydrates, such as having a more highly cross-linked backbone (Versteegh et al. 2012), which explains the generally high preservation potential of dinocysts. These structural differences are unfortunately not resolvable in FTIR spectra. However, the *L. machaerophorum* cysts were analyzed for structural information, which indicated a carbohydrate-based polymer (Versteegh et al. 2012). Due to the strong similarities between *L. machaerophorum* and Group I spectra (Fig. 2), it is probable that the cyst macromolecule is comparable.

Selective preservation of dinocysts in the sedimentary record has been suggested (Zonneveld et al. 1997, 2001, Combourieu-Nebout et al. 1998, McCarthy et al. 2000, Versteegh et al. 2010), although it is likely not a straightforward process (Reichart and

Brinkhuis 2003). Based on these studies peridinialean cysts have been considered more sensitive to oxidation than gonyaulacalean cysts, which is supported by laboratory evidence that peridinialean cysts are destroyed after harsh base treatment (e.g., Dale 1976, Hopkins and McCarthy 2002, Mertens et al. 2009a). However, based on dinosporin composition, we suggest that sensitivity to oxidation is more likely predicated on dinosporin composition. For example, the peridinialean cysts of *P. wisconsinense* withstand treatment by harsh bases during palynological preparation unlike many marine peridinialean species; there are also indications they are more prevalent in sedimentary successions than previously recognized (e.g., Miller et al. 1982, Zippi et al. 1990, McCarthy et al. 2011, Mertens et al. 2012). Therefore, the Group I dinocysts may be inherently more resistant while the Group II dinocysts are more heavily reliant on additional diagenetic factors, such as sulfurization or skeletal rearrangement, to facilitate preservation. It is also interesting to note that the Group II cysts are brown in color, while Group I dinocysts are transparent, suggesting the possibility that the inclusion of nitrogen-containing functional groups and/or different pigments may also contribute to increased oxidative sensitivity. Regardless, these compositional differences between dinosporins provide the first empirical evidence that differences in preservation potential may be related to dinocyst wall chemistry.

## CONCLUSIONS

Micro-FTIR spectroscopic analyses of recent dinoflagellate resting cyst (dinocyst) walls from three large orders (Gonyaulacales, Gymnodiniales, Peridinales) showed that all of the dinocysts had a carbohydrate-based macromolecular composition. Spectral and compositional variability indicated that the cyst wall macromolecule (dinosporin) is a collection of chemically distinguishable but related biomacromolecules determined by the interaction of the cyst-producing dinoflagellate with its environment. Two groups were defined on the basis of this spectral variability. The first group encompasses dinocysts

produced by phototrophic dinoflagellates, which includes all the gonyaulacalean cysts and the peridinialean cysts of *Peridinium wisconsinense*. Their dinosporin has a  $\beta$ -glucan backbone, which concurs with a previous study of *Lingulodinium machaerophorum* (Versteegh et al. 2012). The second group consists of dinocysts produced by heterotrophic dinoflagellates, including the remainder of the peridinialean and all of the gymnodinialean cysts studied. They showed the presence of amide bonds within their dinosporins. These systematic differences reflect the nutritional strategy rather than the phylogeny of the cyst-producing dinoflagellate and may result from the incorporation of chemical compounds in excess within the cell into dinosporins. Thus, the nitrogen-containing functional groups present in the heterotrophic dinosporins may originate from the digestion of prey and the resulting proteinaceous, nitrogen-rich material. The nutritional strategies of the cyst-producing dinoflagellates were polyphyletic, as demonstrated by the grouping of the peridinialean cysts of *P. wisconsinense* with the other phototrophic species (all Gonyaulacales) as well as the grouping of remaining Peridinales and Gymnodinales cysts. As nutritional strategy, not phylogeny, appears to be the primary factor determining dinosporin composition, cyst wall chemistry may show potential as a paleoecological proxy by inferring the past nutritional strategies of extinct taxa. Finally, the compositional differences between the two groups suggest that preservation potential is influenced by dinosporin composition.

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479

## REFERENCES

- Allard, B., Templier, J. & Largeau, C. 1997. Artifactual origin of mycobacterial bacteran. Formation of melanoidin-like artifactual macromolecular material during the usual isolation process. *Org. Geochem.* 26: 691-703.
- Allard, B., Templier, J. & Largeau, C. 1998. An improved method for the isolation of artifact-free algaenans from microalgae. *Org. Geochem.* 28: 543-548.
- Almendros, G., Dorado, J., González-Villa, F. J. & Martin, F. 1997. Pyrolysis of carbohydrate-derived macromolecules: its potential in monitoring the carbohydrate signature of geopolymers. *J. Anal. Appl. Pyrolysis* 40-41: 599-610.
- Arnosti, C. 1995. Measurement of depth- and site-related differences in polysaccharide hydrolysis rates in marine sediments. *Geochim. Cosmochim. Acta* 59: 4247-4257.
- Aspinall, G. O. 1983. The polysaccharides. In Priess, J. [Ed.] *The Biochemistry of Plants*. Academic Press, New York, NY, pp. 473-500.
- Barker, S. A., Bourne, E. J., Stacey, M. & Whiffen, D. H. 1954. Infra-red spectra of carbohydrates. Part I. Some derivatives of D-glucopyranose. *J. Chem. Soc.* 171-176.
- Bauersachs, T., Speelman, E. N., Hopmans, E. C., Reichart, G.-J., Schouten, S. & Sinninghe Damsté, J. S. 2010. Fossilized glycolipids reveal past oceanic N<sub>2</sub> fixation by heterocystous cyanobacteria. *Proc. Natl. Acad. Sci.* 107: 19190-19194.

505 Brenner, W. W. & Biebow, N. 2001. Missing autofluorescence of recent and fossil  
506 dinoflagellate cysts – an indication of heterotrophy? *Neues Jahrb. Mineral. Geol. Palaeontol.*  
507 *Abh. Abt. B.* 219: 229-240.

508

509 Bogus, K., Harding, I. C., King, A., Charles, A. K., Zonneveld, K. & Versteegh, G. J. M.  
510 2012. The composition of species of the *Apectodinium* complex (Dinoflagellata). *Rev. of*  
511 *Palaeobot. Palynol.* 183: 21-31.

512

513 Bouimetarhan, I., Marret, F., Dupont, L. & Zonneveld, K. 2009. Dinoflagellate cyst  
514 distribution in marine surface sediments off West Africa (17-6°N) in relation to sea-surface  
515 conditions, freshwater input and seasonal coastal upwelling. *Mar. Micropaleontol.* 71: 113-  
516 130.

517

518 Bringué, M., Pospelova, V. & Pak, D. 2013. Seasonal production of organic-walled  
519 dinoflagellate cysts in an upwelling system: A sediment trap study from the Santa Barbara  
520 Basin, California. *Mar. Micropaleontol.* 100: 34-51.

521

522 Burton, R. A. & Fincher, G. B. 2009. (1,3; 1,4)- $\beta$ -D-glucans in cell walls of the Poacea, lower  
523 plants and fungi: a tale of two linkages. *Mol. Plant* 2: 873-882.

524

525 Cárdenas, G., Cabrera, G., Taboada, E. & Miranda, S. P. 2004. Chitin characterization by  
526 SEM, FTIR, XRD, and <sup>13</sup>C cross polarization/mass angle spinning NMR. *J. Appl. Polym.*  
527 *Sci.* 93: 1876-1885.

528

529 Chapman, D. V., Dodge, J. D. & Heaney, S. I. 1982. Cyst formation in the freshwater  
530 dinoflagellate *Ceratium hirundinella* (Dinophyceae). *J. Phycol.* 18: 121-129.

531

532 Coates, J. 2000. Interpretation of Infrared Spectra, A Practical Approach. *In* Meyers, R.A.

533 [Ed.] *Encyclopedia of Analytical Chemistry*. John Wiley and Sons Ltd., Chichester, pp.

534 10815-10837.

535

536 Colthup, N. B., Daly, L. H. & Wiberly, S. E. 1990. *Introduction to Infrared and Raman*

537 *Spectroscopy*. Academic Press Limited, London, 282 pp.

538

539 Combourieu-Nebout, N., Paterne, M., Turon, J. L. & Siani, G. 1998. A high-resolution record

540 of the last deglaciation in the central Mediterranean Sea: Palaeovegetation and

541 palaeohydrological evolution. *Quat. Sci. Rev.* 17: 303-317.

542

543 Dale, B. 1976. Cyst formation, sedimentation, and preservation: factors affecting

544 dinoflagellate assemblages in recent sediments from Trondheimsfjord, Norway. *Rev.*

545 *Palaeobot. Palynol.* 22: 39-60.

546

547 Dale, B., Dale, A. L. & Jansen, J. H. F. 2002. Dinoflagellate cysts as environmental indicators

548 in surface sediments from the Congo deep-sea fan and adjacent regions. *Palaeogeogr.*

549 *Palaeoclimatol. Palaeoecol.* 185: 309-338.

550

551 de Leeuw, J. W. 2007. On the origin of sedimentary aliphatic macromolecules: a comment on

552 recent publications by Gupta et al. *Org. Geochem.* 38: 1585-1587.

553

554 de Leeuw, J. W. & Largeau, C. 1993. A review of macromolecular organic compounds that

555 comprise living organisms and their role in kerogen, coal and petroleum formation. *In* Engel,



556 M. H. & Macko, S. A. [Eds.] *Organic Geochemistry: principles and applications*. Plenum  
557 Publishing Corp., New York. pp. 23-72.

558

559 de Leeuw, J. W., Versteegh, G. J. M. & van Bergen, P. F. 2006. Biomacromolecules of algae  
560 and plants and their fossil analogues. *Plant Ecol.* 182: 209-233.

561

562 Elbrächter, M. 1993. *Kolkwitzia* Lindemann 1919 and *Preperidinium* Mangin 1913: correct  
563 genera names in the *Diplopsalis*-group (Dinophyceae). *Nova Hedwigia* 56: 173-178.

564

565 Ellegaard, M. 2000. Variations in dinoflagellate cyst morphology under conditions of  
566 changing salinity during the last 2000 years in the Limfjord, Denmark. *Rev. Palaeobot.*  
567 *Palynol.* 109: 65-81.

568

569 Ellegaard, M., Lewis, J. & Harding, I. 2002. Cyst-theca relationship, life cycle, and effects of  
570 temperature and salinity on the cyst morphology of *Gonyaulax baltica* sp. nov. (Dinophyceae)  
571 from the Baltic Sea area. *J. Phycol.* 38: 775-789.

572

573 Ellegaard, M., Figueroa, R. L. & Versteegh, G. J. M. 2013. Dinoflagellate life cycles, strategy  
574 and diversity: key foci for future research. In Lewis, J. M., Marret, F. & Bradley, L. [Eds.]  
575 *Biological and Geological Perspectives of Dinoflagellates*. The Micropalaeontological Society  
576 Special Publications, Geological Society, London. pp. 249-261.

577

578 Fensome, R. A., Taylor, F. J. R., Norris, G., Sarjeant, W. A. S., Wharton, D. I. & Williams,  
579 G. L. 1993. A classification of fossil and living dinoflagellates. *Micropaleontology Press*  
580 *Special Paper*, 7, pp. 351.

581

582 Frei, E. & Preston, R. D. 1964. Non-cellulosic structural polysaccharides in algal cell walls I.  
583 Xylan in siphonous green algae. *Proc. R. Soc. London, Ser. B.* 160: 293-313.  
584  
585 Fuentes-Grünewald, C., Garcés, E., Rossi, S. & Camp, J. 2009. Use of the dinoflagellate  
586 *Karlodinium veneficum* as a sustainable source of biodiesel production. *J. Ind. Microbiol.*  
587 *Biotechnol.* 36: 1215-1224.  
588  
589 Fuentes-Grünewald, C., Garcés, E., Alacid, E., Sampedro, N., Rossi, S. & Camp, J. 2012.  
590 Improvement in lipid production in the marine strains *Alexandrium minutum* and  
591 *Heterosigma akashiwo* by utilizing abiotic parameters. *J. Ind. Microbiol. Biotechnol.* 39: 207-  
592 216.  
593  
594 Furuhashi, T., Beran, A., Blazso, M., Czegeny, Z., Schwarzing, C. & Steiner, G. 2009.  
595 Pyrolysis GC/MS and IR spectroscopy in chitin analysis of molluscan shells. *Biosci.*  
596 *Biotechnol. Biochem.* 73: 93-103.  
597  
598 Gómez, F. 2012. A quantitative review of the lifestyle, habitat and trophic diversity of  
599 dinoflagellates (Dinoflagellata, Alveolata). *Syst. Biodiversity* 10, 267-275.  
600  
601 González, C., Dupont, L. M., Mertens, K. & Wefer, G. 2008. Reconstructing marine  
602 productivity of the Cariaco Basin during marine isotope stages 3 and 4 using organic-walled  
603 dinoflagellate cysts. *Paleoceanography* 23, PA3215, doi:10.1029/2008PA001602.  
604  
605 Gupta, N. S., Collinson, M. E., Briggs, D. E. G., Evershed, R. P. & Pancost, R. 2006.  
606 Reinvestigation of the occurrence of cutan in plants: implications for the leaf fossil record.  
607 *Paleobiol.* 32: 432-449.

608

609 Hallet, R. I. 1999. Consequences of environmental change on the growth and morphology of

610 *Lingulodinium polyedrum* (Dinophyceae) in culture. Ph.D. dissertation, University of

611 Westminster, London, 109 pp.

612

613 Head, M. J. 1996. Modern dinoflagellate cysts and their biological affinities. *In* Jansonius, J.

614 & McGregor, D. C. [Eds.] *Palynology: Principles and Applications*. AASP Foundation, Salt

615 Lake City, UT, pp. 1197-1248.

616

617 Hemsley, A. R., Barrie, P. J., Scott, A. C. & Chaloner, W.G. 1994. Studies of fossil and

618 modern spore and pollen wall biomacromolecules using  $^{13}\text{C}$  solid state NMR. *In* Eglinton, G.

619 & Kay, R. L. F. [Eds.] *Biomolecular Palaeontology*, NERC Special Publications, 94: 15-19.

620

621 Holzwarth, U., Esper, O. & Zonneveld, K. 2007. Distribution of organic-walled dinoflagellate

622 cysts in sediments of the Benguela upwelling system in relationship to environmental

623 conditions. *Mar. Micropaleontol.* 64: 91-119.

624

625 Holzwarth, U., Esper, O., Zonneveld, K. A. F. 2010. Organic-walled dinoflagellate cysts as

626 indicators of oceanographic conditions and terrigenous input in the NW African upwelling

627 region. *Rev. Palaeobot. Palynol.* 159: 35-55.

628

629 Hopkins, J. A. & McCarthy, F. M. G. 2002. Post-depositional palynomorph degradation in

630 Quaternary shelf sediments: a laboratory experiment studying the effects of progressive

631 oxidation. *Palynol.* 26: 167-184.

632

633 Hoppenrath, M. & Leander, B. S. 2010. Dinoflagellate Phylogeny as inferred from heat shock  
634 protein 90 and ribosomal gene sequences. *PLoS ONE* 5: e13220.

635

636 Jacobson, D. M. & Anderson, D. M. 1986. Thecate heterotrophic dinoflagellates: feeding  
637 behavior and mechanisms. *J. Phycol.* 22: 249-258.

638

639 Kačuráková, M. & Wilson, R.H. 2001. Developments in mid-infrared FT-IR spectroscopy of  
640 selected carbohydrates. *Carbohydr. Polym.* 44: 291-303.

641

642 Kačuráková, M., Wellner, N., Ebringerová, A., Hromádková, Z., Wilson, R. H. & Belton, P.  
643 S. 1999. Characterisation of xylan-type polysaccharides and associated cell wall components  
644 by FT-IR and FT-Raman spectroscopies. *Food Hydrocolloids* 13: 35-41.

645

646 Kok, M. D., Schouten, S. & Sinninghe Damsté, J. S. 2000. Formation of insoluble,  
647 nonhydrolyzable, sulfur-rich macromolecules via incorporation of inorganic sulfur species  
648 into algal carbohydrates. *Geochim. Cosmochim. Acta* 64: 2689-2699.

649

650 Kokinos, J. P. & Anderson, D. M. 1995. Morphological development of resting cysts in  
651 culture of the marine dinoflagellate *Lingulodinium polyedrum* (= *L. machaerophorum*).  
652 *Palynol.* 19: 143-165.

653

654 Kokinos, J. P., Eglinton, T. I., Goñi, M. A., Boon, J. J., Martoglio P. A. & Anderson, D. M.  
655 1998. Characterization of a highly resistant biomacromolecular material in the cell wall of a  
656 marine dinoflagellate resting cyst. *Org. Geochem.* 28: 265-288.

657

658 Kouli, K., Brinkhuis, H. & Dale, B. 2001. *Spiniferites cruciformis*: a fresh water  
659 dinoflagellate cyst? *Rev. Palaeobot. Palynol.* 113: 273-286.  
660

661 Kurita, K. 2006. Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar.*  
662 *Biotechnol.* 8: 203–226.  
663

664 Lechien, V., Rodriguez, C., Ongena, M., Hilgsmann, S., Rulmont, A. & Thonart, P. 2006.  
665 Physiochemical and biochemical characterization of non-biodegradable cellulose in Miocene  
666 gymnosperm wood from the Entre-Sambre-et-Meuse, Southern Belgium. *Org. Geochem.* 37:  
667 1465-1476.  
668

669 Leroy, S. A. G., Boyraz, S. & Gürbüz, A. 2009. High-resolution palynological analysis in  
670 Lake Sapanca as a tool to detect recent earthquakes on the North Anatolian Fault. *Quat. Sci.*  
671 *Rev.* 28: 2616-2632.  
672

673 Lessard, E. J. & Swift, E. 1986. Dinoflagellates from the North Atlantic classified as  
674 phototrophic or heterotrophic by epifluorescence microscopy. *J. Plankton Res.* 8: 1209-1215.  
675

676 Lewis, J., Rochon, A., Ellegaard, M., Mudie, P. J. & Harding, I. C. 2001. The cyst-theca  
677 relationship of *Bitectatodinium tepikiense* (Dinophyceae). *Eur. J. Phycol.* 36: 137-146.  
678

679 Marchand, C., Disnar, J. R., Lallier-Vergès, E. & Lottier, N. 2005. Early diagenesis of  
680 carbohydrates and lignin in mangrove sediments subject to variable redox conditions (French  
681 Guiana). *Geochim. Cosmochim. Acta* 69: 131-142.  
682

683 Matsuoka, K. 1985. Cyst and thecate forms of *Pyrophacus steinii* (Schiller) Wall et Dale,  
684 1971. *Transactions and proceedings of the Palaeontological Society of Japan, New series.*  
685 140: 240-262.

686

687 Matsuoka, K. 1988. Cyst-theca relationships in the Diplopsalid group (Peridiniales,  
688 Dinophyceae). *Rev. Palaeobot. Palynol.* 56: 95-122.

689

690 Matsuoka, K., McMinn, A. & Wrenn, J. H. 1997. Restudy of the holotype of *Operculodinium*  
691 *centrocarpum* (Deflandre & Cookson) Wall (Dinophyceae) from the Miocene of Australia,  
692 and the taxonomy of related species. *Palynol.* 21: 19-33.

693

694 Matsuoka, K., Cho, H.-J. & Jacobson, D. M. 2000. Observations of the feeding behavior and  
695 growth rates of the heterotrophic dinoflagellate *Polykrikos kofoidii* (Polykrikaceae,  
696 Dinophyceae). *Phycologia* 39: 82-86.

697

698 Matsuoka, K., Kawami, H., Nagai, S., Iwataki, M. & Takayama, H. 2009. Re-examination of  
699 cyst-motile relationships of *Polykrikos kofoidii* Chatton and *Polykrikos schwartzii* Bütschli  
700 (Gymnodiniales, Dinophyceae). *Rev. Palaeobot. Palynol.* 154: 79-90.

701

702 Matthiessen, J., de Vernal, A., Head, M., Okolodkov, Y., Zonneveld, K. A. F. & Harland, R.  
703 2005. Modern organic-walled dinoflagellate cysts in Arctic marine environments and their  
704 (paleo-) environmental significance. *Palaeontolog. Z.* 79: 3-51.

705

706 McCarthy, F. M. G., Gostlin, K. E., Mudie, P. J. & Scott, D.B. 2000. Synchronous  
707 palynological changes in early Pleistocene sediments off New Jersey and Iberia, and a  
708 possible paleoceanographic explanation. *Palynol.* 24: 63-77.

709

710 McCarthy, F. M. G, Mertens, K. N., Ellegaard, M., Sherman, K., Pospelova, V., Ribeiro, S.,  
711 Blasco, S. & Vercauteren, D. 2011. Resting cysts of freshwater dinoflagellates in southeastern  
712 Georgian Bay (Lake Huron) as proxies of cultural eutrophication. *Rev. Palaeobot. Palynol.*  
713 166: 46-62.

714

715 Menden-Deuer, S., Lessard, E. J., Satterberg, J. & Grünbaum, D. 2005. Growth and starvation  
716 survival capacity of three species of the pallium feeding thecate dinoflagellate genus  
717 *Protoperidinium* (Peridiniacea, Dinophyceae) distributions. *Aquat. Microbial Ecol.* 41: 145-  
718 152.

719

720 Mertens, K. N., Verhoeven, K., Verleye, T., Louwye, S., Amorim, A., Ribeiro, S., Deaf, A.  
721 S., Harding, I. C., De Schepper, S., González, C., Kodrans-Nsiah, M., de Vernal, A., Henry,  
722 M., Radi, T., Dybkjaer, K., Poulsen, N. E., Feist-Burkhardt, S., Chitolie, J., Heilmann-  
723 Clausen, C., Londeix, L., Turon, J.-L., Marret, F., Matthiessen, J., McCarthy, F. M. G.,  
724 Prasad, V., Pospelova, V., Kyffin Hughes, J. E., Riding, J. B., Rochon, A., Sangiorgi, F.,  
725 Welters, N., Sinclair, N., Thun, C., Soliman, A., van Nieuwenhove, N., Vink A. & Young, M.  
726 2009a. Determining the absolute abundance of dinoflagellate cysts in recent marine  
727 sediments: The *Lycopodium* marker-grain method put to the test. *Rev. Palaeobot. Palynol.*  
728 157: 238-252.

729

730 Mertens, K. N., Ribeiro, S., Bouimetarhan, I., Caner, H., Combourieu-Nebout, N., Dale, B.,  
731 de Vernal, A., Ellegaard, M., Filipova, M., Godhe, A., Goubert, E., Grøsfjeld, K., Holzwarth,  
732 U., Kotthoff, U., Leroy, S. A. G., Londeix, L., Marret, F., Matsuoka, K., Mudie, P. J., Naudts,  
733 L., Peña-Manjarrez, J. L., Persson, A., Popescu, S. M., Pospelova, V., Sangiorgi, F., van der  
734 Meer, M. T. J., Vink A., Zonneveld, K. A. F., Vercauteren, D., Vlassenbroeck, J. & Louwye,

735 S., 2009b. Process length variation in cysts of a dinoflagellate, *Lingulodinium*  
736 *machaerophorum*, in surface sediments: investigating its potential as salinity proxy. *Mar.*  
737 *Micropal.* 70: 54-69.

738

739 Mertens, K. N., Rengefors, K., Moestrup, Ø. & Ellegaard, M. 2012. A review of recent  
740 freshwater dinoflagellate cysts: taxonomy, phylogeny, ecology and palaeocology. *Phycologia*  
741 51: 612-619.

742

743 Miller, A. A. L., Mudie, P. J. & Scott, D. B. 1982. Holocene history of Bedford Basin, Nova  
744 Scotia: foraminifera, dinoflagellate and pollen records. *Can. J. Earth Sci.* 19: 2342-2367.

745

746 Moers, M. E. C., de Leeuw, J. W. & Baas, M. 1994. Origin and diagenesis of carbohydrates  
747 in ancient sediments. *Org. Geochem.* 21: 1093-1106.

748

749 Naustvoll, L.-J. 2000. Prey size spectra and food preferences in thecate heterotrophic  
750 dinoflagellates. *Phycologia* 39: 187-198.

751

752 Nevo, Z. & Sharon, N. 1969. The cell wall of *Peridinium westii*, a non cellulosic glucan.  
753 *Biochim. Biophys. Acta* 173: 161-175.

754

755 Orr, R. J. S., Murray, S. A., Stüken, A., Rhodes, L. & Jakobsen, K. S. 2012. When naked  
756 became armored: An eight-gene phylogeny reveals monophyletic origin of theca in  
757 dinoflagellates. *PLoS ONE* 7: e50004.

758

759 Pandey, K. K. 1999. A study of chemical structure of soft and hardwood and wood polymers  
760 by FTIR spectroscopy. *J. Appl. Polym. Sci.* 71: 1969-1975.



761

762 Pfiester, L. A. & Anderson, D. M. 1987. Dinoflagellate reproduction. *In* Taylor, F. J. R. [Ed.]

763 *The Biology of Dinoflagellates*. Blackwell Scientific, Oxford, pp. 611-648.

764

765 Pospelova, V., Pedersen, T. F. & de Vernal, A. 2006. Dinoflagellate cysts as indicators of

766 climatic and oceanographic changes during the past 40 kyr in the Santa Barbara Basin,

767 southern California. *Paleoceanography* 21, PA2010, doi: 10.1029/2005PA001251.

768

769 Pospelova, V., de Vernal, A. & Pedersen, T. F. 2008. Distribution of dinoflagellate cysts in

770 surface sediments from the northeastern Pacific Ocean (43-25°N) in relation to sea-surface

771 temperature, salinity, productivity and coastal upwelling. *Mar. Micropal.* 68: 21-48.

772

773 Reichart, G. J. & Brinkhuis, H. 2003. Late Quaternary *Protoperidinium* cysts as indicators of

774 paleoproductivity in the northern Arabian Sea. *Mar. Micropal.* 49: 303-315.

775

776 Rochon, A., de Vernal, A., Turon, J. L., Matthiessen, J. & Head, M. J. 1999. Distribution of

777 recent dinoflagellate cysts in surface sediments from the North Atlantic Ocean and adjacent

778 seas in relation to sea-surface parameters. *American Association of Stratigraphic*

779 *Palynologists Foundation, Contribution series 35*, Dallas, TX, pp. 152.

780

781 Šandula, J., Kogan, G., Kačuráková, M. & Machová, E. 1999. Microbial (1-3)- $\beta$ -D-glucans,

782 their preparation, physico-chemical characterization and immunomodulatory activity.

783 *Carbohydr. Polym.* 38: 247-253.

784

785 Schnepf, E. & Elbrächter, M. 1992. Nutritional strategies in dinoflagellates: A review with

786 emphasis on cell biological aspects. *Eur. J. Protistol.* 28: 3-24.

787

788 Sekida, S., Horiguchi, T. & Okuda, K. 2004. Development of thecal plates and pellicle in the  
789 dinoflagellate *Scrippsiella hexapraeicingula* (Peridinales, Dinophyceae) elucidated by  
790 changes in stainability of the associated membranes. *Eur. J. Phycol.* 39: 105-114.

791

792 Stankiewicz, B. A., Mastalerz, M., Hof, C. H. J., Bierstedt, A., Flannery, M. B., Briggs, D. E.  
793 G. & Evershed, R. P. 1998. Biodegradation of the chitin-protein complex in crustacean  
794 cuticle. *Org. Geochem.* 28: 67-76.

795

796 Stone, B. A. 2009. Chemistry of  $\beta$ -glucans. In Bacic, A., Fincher, G. B. & Stone, B. A. [Eds.]  
797 *Chemistry, biochemistry, and biology of (1-3)- $\beta$ -glucans and related polysaccharides*.  
798 Academic Press, Elsevier Inc., London, pp. 5-46.

799

800 Tardio, M., Sangiorgi, F., Ellegaard, M., Di Giuseppe, G., Filippi, M.L., Cantonati, M. &  
801 Lotter, A. F. 2006. Peridinioid dinoflagellate cysts in a Holocene high-mountain lake deposits  
802 in Italy. *J. Paleolimnol.* 36: 315-318.

803

804 Taylor, F. J. R., 1987. Ecology of dinoflagellates: general and marine ecosystems. In Taylor,  
805 F. J. R. [Ed.] *The Biology of Dinoflagellates*. Botanical Monographs 21, Oxford, pp. 398-502.

806

807 Taylor, F.J.R. 2004. Illumination or confusion? Dinoflagellate molecular phylogenetic data  
808 viewed from a primarily morphological standpoint. *Phycol. Res.* 52: 308-324.

809

810 Thornton, D. C. O., Santillo, D. & Thake, B. 1999. Prediction of sporadic mucilaginous algal  
811 blooms in the northern Adriatic Sea. *Mar. Poll. Bull.* 38: 891-898.

812

813 van Dongen, B. E., Schouten, S., Baas, M., Geenevasen, J. A. J. & Sinninghe Damsté, J. S.  
 814 2003. An experimental study of the low-temperature sulfurization of carbohydrates. *Org.*  
 815 *Geochem.* 34: 1129-1144.  
 816  
 817 Venyaminov, S. Yu. & Kalnin, N.N. 1990. Quantitative IF spectrophotometry of peptide  
 818 compounds in water (H<sub>2</sub>O) solutions, I. Spectral parameters of amino acid residue absorption  
 819 bands. *Biopolym.* 30: 1243-1257.  
 820  
 821 Verleye, T. J., Pospelova, V., Mertens, K. N. & Louwye, S. 2011. The geographical  
 822 distribution and (palaeo) ecology of *Selenopemphix undulata* sp. nov., a late Quaternary  
 823 dinoflagellate cyst from the Pacific Ocean. *Mar. Micropal.* 78: 65-83.  
 824  
 825 Versteegh, G. J. M., Blokker, P., Wood, G., Collinson, M. E., Sinninghe Damsté, J. S. & de  
 826 Leeuw, J. W. 2004. An example of oxidative polymerization of unsaturated fatty acids as a  
 827 preservation pathway for dinoflagellate organic matter. *Org. Geochem.* 35: 1129-1139.  
 828  
 829 Versteegh, G. J. M., Blokker, P., Marshall, C. P. & Pross, J. 2007. Macromolecular  
 830 composition of the dinoflagellate cyst *Thalassiphora pelagica* (Oligocene, SW Germany).  
 831 *Org. Geochem.* 38: 1643-1656.  
 832  
 833 Versteegh, G. J. M., Zonneveld, K. & de Lange, G. J. 2010. Selective aerobic and anaerobic  
 834 degradation of lipids and palynomorphs in the Eastern Mediterranean since the onset of  
 835 sapropel S1 deposition. *Mar. Geol.* 278: 177-192.  
 836  
 837 Versteegh, G. J. M., Blokker, P., Bogus, K., Harding, I. C., Lewis, J., Oltmanns, S., Rochon,  
 838 A. & Zonneveld, K. A. F. 2012. Flash pyrolysis and infrared spectroscopy of cultured and

839 sediment derived *Lingulodinium polyedrum* (Dinoflagellata) cyst walls. *Org. Geochem.* 43:  
840 92-102.

841

842 Wall, D. & Dale, B. 1968. Modern dinoflagellate cysts and evolution of the Peridinales.  
843 *Micropaleontol.* 14: 265–304.

844

845 Wotton, R. S. 2004. The ubiquity and many roles of exopolymers (EPS) in aquatic systems.  
846 *Sci. Mar.* 68 (Suppl.): 13-21.

847

848 Yuen, S. N., Choi, S.-M., Phillips, D. E. & Ma, C.-Y. 2009. Raman and FTIR spectroscopic  
849 study of carboxymethylated non-starch polysaccharides. *Food Chem.* 114: 1091-1098.

850

851 Zhang, H., Bhattacharya, D. & Lin, S. 2007. A three-gene dinoflagellate phylogeny suggests  
852 monophyly of Prorocentrales and a basal position for Amphidinium and Heterocapsa. *J. Mol.*  
853 *Evol.* 65: 463-474.

854

855 Zippi, P., Yung, Y.-K., McAndrews, J. A., Norris, G. & Welbourn, P. 1990. An investigation  
856 of the potential of zygmematacean zygospores, Peridinium, and Pediatrum as paleo-  
857 indicators of recent lake acidification. *Environmental Research, Technology Transfer*  
858 *Conference (Toronto, Canada) Proceedings*, 1, pp. 393-396.

859

860 Zonneveld, K. A. F. & Susek, E. 2007. Effects of temperature, light and salinity on cyst  
861 production and morphology of *Tuberculodinium vancampoe* (the resting cyst of *Pyrophacus*  
862 *steinii*). *Rev. Palaeobot. Palynol.* 145: 77-88.

863

864 Zonneveld, K. A. F., Versteegh, G. J. M., & de Lange, G.J. 1997. Preservation of organic-  
865 walled dinoflagellate cysts in different oxygen regimes: a 10,000 year natural experiment.  
866 *Mar. Micropaleontol.* 29: 393-405.

867

868 Zonneveld, K. A. F., Versteegh, G. J. M. & de Lange, G. J. 2001. Palaeoproductivity and  
869 post-depositional aerobic organic matter decay reflected by dinoflagellate cyst assemblages of  
870 the Eastern Mediterranean S1 sapropel. *Mar. Geol.* 172: 181-195.

871

872 Zonneveld, K. A. F., Marret, F., Versteegh, G. J. M., Bogus, K., Bonnet, S., Bouimetarhan, I.,  
873 Crouch, E., de Vernal, A., Elshanawany, R., Edwards, L., Esper, O., Forke, S., Grøsfjeld, K.,  
874 Henry, M., Holzwarth, U., Kieft, J.-F., Kim, S.-Y., Ladouceur, S., Ledu, D., Chen, L.,  
875 Limoges, A., Londeix, L., Lu, S.-H., Mahmoud, M., Marino, G., Matsuoka, K., Matthiessen,  
876 J., Mildenhall, D. C., Mudie, P., Neil, H. L., Pospelova, V., Qi, Y., Radi, T., Richerol, T.,  
877 Rochon, A., Sangiorgi, F., Solignac, S., Turon, J.-L., Verleye, T., Wang, Y. & Young, M.  
878 2013. Atlas of modern dinoflagellate cyst distribution based on 2405 data points. *Rev.*  
879 *Palaeobot. Palynol.* 191: 1-197.

880

881 *Tables*

882 Table 1: Surface sediment and culture sample information from which the dinocyst specimens

883 were isolated.

#	Name	Location	Latitude	Longitude	Water depth (m)	Setting	Reference
1	GeoB 2341	Benguela upwelling	31°55'48"S	18°12'36"E	84	Marine	Holzwarth et al. (2007)
2	GeoB 4804	Benguela upwelling	24°8'60"S	12°40'12"E	2090	Marine	Holzwarth et al. (2007)
3	GeoB 6010	NE Atlantic (off NW Africa)	30°15'N	10°2'16.8"W	406	Marine	Holzwarth et al. (2010)
4	SV5-C	Honey Harbour (Lake Huron, Canada)	44°52'26"N	79°48'55"W	19.4	Lacustrine	McCarthy et al. (2011)
5	Schillig	Wadden Sea (NW Germany)	53°42'56"N	7°58'11.93"E	0.5	Culture	-
6	GeoB 1010	Omura Bay (Kyushu, Japan)	33°N	128°49'48"E	Not stated	Culture	Zonneveld and Susek (2007)

884

885

886 Table 2: Dinocyst species analyzed in this study, their motile affinities, and nutritional  
887 strategy with reference to the respective studies.

Sample*	Specimens measured (#)	Dinocyst species	Motile affinity	Order
2	3	<i>Impagidinium patulum</i> (Wall) Stover and Evitt	<i>Gonyaulax</i> sp. Diesing <sup>a</sup>	Gonyaulacales
2,3	6 (3 from each location)	<i>Operculodinium centrocarpum</i> sensu Wall and Dale	<i>Protoceratium reticulatum</i> (Claparède <i>et</i> Lachmann) Bütschli <sup>b</sup>	Gonyaulacales
2	4	<i>Spiniferites pachydermus</i> (Rossignol) Reid	<i>Gonyaulax</i> sp. Diesing <sup>c</sup>	Gonyaulacales
6	3	<i>Tuberculodinium vancampoe</i> (Rossignol) Wall	<i>Pyrophacus steinii</i> (Schiller) Wall and Dale <sup>d</sup>	Gonyaulacales
4	3	Cyst of <i>Peridinium wisconsinense</i> Eddy	<i>Peridinium wisconsinense</i> Eddy <sup>e</sup>	Peridiniales
1	4	<i>Brigantedinium</i> spp. Reid	<i>Protoperidinium</i> sp. Bergh <sup>c</sup>	Peridiniales
5	3	<i>Dubridinium caperatum</i> Reid	<i>Preperidinium meunieri</i> (Pavillard) Elbrächter <sup>f</sup>	Peridiniales
1	4	Cyst of <i>Polykrikos kofoidii</i> Chatton	<i>Polykrikos kofoidii</i> Chatton <sup>g</sup>	Gymnodiniales
1,2	6 (3 from each location)	Cyst of <i>Polykrikos schwartzii</i> Bütschli	<i>Polykrikos schwartzii</i> Bütschli <sup>g</sup>	Gymnodiniales

888 \*Sample numbers correspond to Table 1. <sup>a</sup>Rochon et al. (1999). <sup>b</sup>Matsuoka et al. (1997).

889 <sup>c</sup>This study. <sup>d</sup>Matsuoka (1985). <sup>e</sup>McCarthy et al. (2011). <sup>f</sup>Matsuoka (1988); see Elbrächter  
890 (1993) for synonymy. <sup>g</sup>Matsuoka et al. (2009). <sup>h</sup>Gómez (2012).

891

892

893 Table 3: Assignments of major FTIR absorptions present in Group I (phototrophic) and  
 894 Group II (heterotrophic) dinosporins. See Table 2 for each species' nutritional strategy.

Dinocysts	Wavenumber (cm <sup>-1</sup> )	Assignment	Comments
<b><u>Group I</u></b>			
	3348	$\nu\text{OH}$	
	2925	$\nu\text{CH}$	
	2860	$\nu\text{CH}$	
	1640	$\nu\text{C=O}$	
	1600	$\nu\text{C=C} + \nu\text{C=O}$	
	1430	$\delta\text{CH}_2$	
	1370	$\delta\text{CH} + \delta\text{C-CH}_3$	
	1318	$\delta\text{OH}$	
	1163	$\nu\text{C-O-C}$	
	1112	$\nu\text{C-O}$	Glucose ring
	1059	$\nu\text{C-O}$	
	1033	$\nu\text{C-O}$	
	897	$\gamma\text{CH}$	$\beta$ -glycosidic bond
<b><u>Group II</u></b>			
	3479, 3448, 3426	$\nu\text{OH}$	
	3268	$\nu\text{NH}$	
	3106	$\nu\text{NH}$	
	2965-2860	$\nu\text{CH}$	
	1660, 1627	$\nu\text{C=O}$	Amide I
	1585-1550	$\nu\text{CN} + \delta\text{NH}$	Amide II
	1420	$\delta\text{CH}_2$	



	1370	$\delta\text{CH} + \delta\text{C-CH}_3$	
	1312	$\nu\text{CN} + \delta\text{NH}$	Amide III
	1255	$\delta\text{NH}$	
	1157	$\nu\text{C-O-C}$	Ring
	1113	$\nu\text{C-O}$	Glucose ring
	1060	$\nu\text{C-O}$	
	1027	$\nu\text{C-O}$	
	896, 902	$\gamma\text{CH}$	$\beta$ -glycosidic bond
	875-746	$\rho\text{CH}_2$	
	698	$\gamma\text{NH}$	Amide V

895

896

897 *Figure captions*

898 Figure 1: Locations of the marine and lacustrine surface samples analyzed in this study.

899

900 Figure 2: FTIR spectra of the Group I (phototrophic) dinocysts compared to *Lingulodinium*  
901 *machaerophorum* from sediment (black line) and culture (gray line; Versteegh et al. 2012)  
902 and cellulose (Pandey 1999). The gray *O. centrocarpum* spectrum denotes the analysis from  
903 the second measured location (see Tables 1, 2). Absorption assignments are given in Table 3.

904

905 Figure 3: FTIR spectra of the Group II (heterotrophic) dinocysts and the chitin standard. The  
906 gray line for cysts of *P. schwartzii* reflects spectral data from a second location (see Tables 1,  
907 2). Absorption assignments are given in Table 3.

908

909 Figure 4: Phylogenetic subdivision of the major orders according to phylogenies proposed by  
910 Fensome et al. (1993) and Taylor (2004) and comparison with the two groups derived from  
911 micro-FTIR measurements.

912